Hereditary Evaluation of Legg-Calve-Perthes Disease in the Miniature Pinscher

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Summary

A hereditary evaluation of Legg-Calve-Perthes Disease (LCPD) in the Miniature Pinscher (Min Pin) is proposed to assess the genetic contribution to disease presentation. Initial analyses include complex segregation analyses (CSA) to determine the mode of transmission of LCPD in the Min Pin, as well as heritability analyses to estimate the contribution of genetic and non-genetic (environmental) factors in the total population's phenotypic variance. Future studies are dependent on the results of the CSA: simple patterns of inheritance (*i.e.*, autosomal recessive, autosomal dominant) require fewer dogs in association studies than complex traits (*i.e.*, polygenic/multifactoral). Linkage studies (linkage analysis; linkage disequilibrium) draw on basic biological principles in that markers, or genetic "signposts" (*e.g.*, microsatellites, single nucleotide polymorphisms (SNPs)) located near a disease-controlling gene will be co-inherited, or linked, with the disease phenotype.

To determine linkage, there are two approaches we can take: classical linkage analysis (LA) which traces alleles through generations, and linkage disequilibrium (LD), which utilizes unrelated dogs that show the same phenotype.

Linkage analysis requires an extensive pedigree (3+ generations, all siblings in a litter represented) with informative matings (those that produce disease), and samples taken from as many individuals as possible from the constructed pedigree. The inheritance of alleles associated with a particular trait is then traced through the pedigree. Microsatellite markers (short, repetitive sequences of variable length dispersed throughout the genome) from the canine genome have been used in various studies from our lab. Two genes or markers that are very close are less likely to be separated by naturally occurring recombination than two genes or markers at opposite ends of a chromosome. Linkage analysis looks for a particular allele that is always or most often associated with the disease status. Alleles with high correlation to a particular phenotype are considered linked, and the area of the chromosome is then examined in order to identify candidate genes.

Linkage disequilibrium (LD) is based on similar principles but does not require tracing of alleles through pedigrees. LD relies on the principle that unrelated dogs of a particular breed, sharing the same disease phenotype, will share the same defective gene causative for the disease, and that disease can be traced to a common ancestor. For LD, disease allele(s) must be shown to correlate with the affected phenotype in many different pedigrees. Also, no individual in the analysis can be related to any other individual by

three generations in order to show conservation of the phenotype. The LD analysis looks for an association of one allele with affected individuals.

Microsatellite markers can be used for LD studies, but the newest technology available for canine genetics research is an oligonucleotide-based array that identifies the DNA sequence at specific loci throughout the genome. The sequences at these loci are known to vary by a single base pair. A large-scale study of the canine genome has identified more than 25,000 informative SNPs across breeds that are useful in the identification of disease-causing genes. Approximately 40 unrelated dogs (20 affected and 20 unaffected) are required to complete a SNP analysis for an autosomal recessive trait, 100 unrelated dogs (50 affected and 50 unaffected) are required for an autosomal dominant trait, and 200 unrelated dogs (100 affected and 100 unaffected) are required for complex traits.

We are requesting $2000 to compensate lab personnel for collating of materials, construction of a database and pedigrees, and analysis of all data.

Funds for continuing experiments after the CSA would be discussed at the completion of these initial analyses provided the data indicate a strong genetic basis of disease and encourage us to move forward with the project. If the disease is judged to be too complex (if a polygenic mode of inheritance with no major gene is identified), the project will be re-evaluated as to whether it is prudent to continue research when no results may be produced – especially considering the funding could be applied to other worthwhile research endeavors.

Following this summary, we have a grant proposal written in standard scientific format for the initial phase of research (heritability and CSA).

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Amount requested: $2,000

Grant period: October 1, 2007- January 31, 2008

Scientific Abstract
Legg-Calve-Perthes Disease (LCPD) is a debilitating developmental disease that affects toy and miniature breeds of dog. The only easily observable indications of this condition are pain, lameness, and muscle atrophy of the hip joint. These signs are not exclusive to LCPD, and are often attributed to minor trauma during the early stages of disease. LCPD is diagnosed primarily by radiographic changes in the coxofemoral joint, with patient breed and age as additional factors in diagnosis. Due to the developmental nature and the unknown etiology of the disease, LCPD is difficult to predict and prevent. Surgical intervention provides the best prognosis for the dog, but places significant financial obligation on the owner. Identification of a mode of transmission and an estimate of heritability in the Miniature Pinscher (Min Pin) is proposed in this pilot study of LCPD.

Lay Abstract
Legg-Calve-Perthes Disease (LCPD) is a debilitating developmental disease that affects toy and miniature breeds of dog. Pain, lameness, and muscle atrophy of the hip joint are the only easily observable indications of the condition, and are sometimes attributed to minor trauma. LCPD is diagnosed by examining x-rays (radiographs) of affected dogs. LCPD is difficult to predict and prevent, but good or excellent quality of life can be acquired with surgery. Identifying the inheritance pattern of LCPD and the degree to which genetics govern the disease (heritability) are planned for the Miniature Pinscher (Min Pin).

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Significance of Research
LCPD is an orthopedic abnormality affecting the femoral head and neck of puppies. The occurrence of LCPD is primarily in toy and miniature breeds of dog (those <20 pounds), with a higher predominance in the Miniature Pincher, Pug, Yorkshire Terrier, and West Highland White Terrier (LaFond et al. 2002).

The initial phase of the investigation of LCPD in the Min Pin is to identify the mode of transmission and calculate estimates of heritability. Elucidating the basis of inheritance is critical for developing sound breeding practices and minimizing the number of cases of LCPD in these breeds.

Background of Research
Legg-Calve-Perthes Disease (LCPD) is a developmental disease characterized by avascular necrosis of the femoral head and neck, first described in veterinary literature independently by Spicer, Schnelle, and Moltzen-Nielsen in the late 1930's (Ljunggren 1967). This disease primarily affects toy and miniature breeds of dog, with an age of

onset between 4 and 11 months of age. There is no predilection to one sex, and it is generally accepted to be an autosomal recessive condition (Demko and McLaughlin, 2005). This condition can affect either one or both of the femurs (unilateral or bilateral) with only 10-15% of cases having bilateral involvement. There are no reports of one side being more commonly affected than the other. The exact cause of LCPD is unknown, but the disease presentation classically results in osteonecrosis of the femoral head secondary to ischemia or vascular insult. While LCPD is known to have genetic involvement, the extent to which genetics play a role in disease presentation, and what additional environmental factors contribute to the disease, remain unclear.

LCPD is diagnosed by a combination of dog history (*i.e*., age, breed, clinical signs), physical examination, histology, and radiographic evidence of structural femoral changes. The treatment and prognosis of LCPD varies. Surgical intervention, primarily femoral head ostectomy (FHO), has a greater than 84% success rate of alleviating pain and lameness. Conservative treatments, comprised of rest, restricted exercise, proper nutrition, joint stabilization techniques and analgesics, rarely improve clinical signs. Less than 25% of cases opting for conservative treatment result in resolution of pain and limping. (Demko and McLaughlin 2005)

While surgical intervention has better odds for alleviating and resolving the clinical signs of LCPD, it also places a financial burden on owners of affected dogs. FHO procedures cost a minimum of $1000, not including any physical therapy and recheck examination fees during the three to six month recovery period. Within the last few years, total hip replacement surgery has become available to toy breeds. This procedure reconstructs the coxofemoral joint, making it the “gold standard” in surgical LCPD repair. The cost of hip replacement surgery is estimated to be $5000, without factoring in additional follow-up therapies and examinations.

LCPD has been researched in various studies to determine the mode of inheritance and etiological factors contributing to the disease in multiple breeds: Manchester Terrier, Yorkshire Terrier, Miniature Poodle, and Pug. The proposed modes of inheritance for these dogs are: multi-factoral, monogenic autosomal recessive (AR), monogenic AR, and monogenic AR, respectively (Vasseur et al. 1988, Robinson 1992). Analysis of litter data from Min Pin puppies affected by LCPD found an incidence of disease suggestive of a monogenic autosomal recessive mode of inheritance (Robinson 1992). No multi-generational pedigree analyses or complex segregation analyses (CSA) have been published for the Min Pin. Future studies of LCPD in the Min Pin will be determined based on the results of these inheritance studies.

Specific Objectives

(1) Construct multi-generational pedigrees from Min Pin information received
(2) Complete estimate of heritability and CSA for Min Pin pedigrees

Description of Research

Estimate of heritability
A threshold model for the liability to disease will be implemented to estimate the heritability of LCPD in the Min Pin. This strategy has been recently employed in the evaluation of a developmental disease in the Havanese (Starr et al. 2007). Calculations will be carried out using Sequential Oligogenic Linkage Analysis Routines (SOLAR) (Almasy and Blangero 1998, Blangero et al. 2005), making use of the approach documented by Duggirala et al. (1997).

Complex segregation analysis
Complex segregation analysis, developed by Bonney (1986), is intended to integrate Mendelian transmission genetics at a single locus with the patterns of covariance expected in polygenic inheritance. Lynch and Walsh (1998) provide a more complete description of complex segregation analysis and the methods used in this investigation will follow those employed in Starr et al. (2007). Evaluation of the models necessary for complex segregation analysis will be conducted with the Bayesian software package iBay (2006, version 1.0). The iBay software is an extension of MaGGic (Janss 1998) rewritten to accommodate complex segregation analysis in binary traits, as well as normally distributed phenotypes, for pedigrees that include inbreeding. In addition, a test of the effect of gender on the predisposition to disease will be tested using a likelihood ratio test.

Expected outcomes & potential applications
We expect to identify the mode of transmission of LCPD in the Min Pin and estimate the degree to which genetics play a role in the disease presentation. This will be accomplished by collecting and analyzing pedigree information from multiple families of dogs. Elucidating the inheritance of LCPD in the Min Pin will provide concrete information that breeders can incorporate into their breeding decisions. Any insights gained from the Min Pin research may be applicable to other toy or miniature breeds affected by LCPD.

Anticipated problems
The only anticipated problem is the construction of informative pedigrees with detailed phenotypic information. A minimum of 3 generations and the segregation of LCPD are required for the pedigree analysis. Collecting samples and assembling the pedigree information for a sufficient amount of dogs will entail the largest commitment of time. It should be noted that because the data come from owner submissions, the data will be collected in a non-random fashion. Moreover, as this is a study of inheritance, the data will be constructed around probands. The analysis methods proposed in this grant, however, take into account the ascertainment bias given the dogs used in the analysis are considered random samples of Min Pins.

References

Almasy L, Blangero J, 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet. 62:1198-1211.

Blangero J, Almasy L, Goring H, Williams J, Dyer T, Peterson C, 2005. Sequential oligogenic linkage analysis routines (SOLAR). 3.0.4 edition.

Bonney GE, 1986. Regressive logistic models for familial disease and other binary traits. Biometrics. 42:611-625.

Demko J and McLaughlin R, Developmental Orthopedic Disease, Vet Clin Small Anim (2005) 35:1111-1135.

Duggirala R, Williams JT, Williams-Blangero S, Blangero J, 1997. A variance component approach to dichotomous trait linkage analysis using a threshold model. Genet Epidemiol. 14:987-992.

Janss LLG, 1998. MAGGIC: A package of subroutines for genetic analyses with Gibbs sampling. In: Proceedings of the 6th World Congress on Genetics Applied to Livestock Production: vol 27. Organizing Committee on 6th WCGLAP, Armidale, Australia. p. 459-460

LaFond E, Breur GJ, Austin CC, 2002. Breed susceptibility for developmental orthopedic diseases in dogs. J Am Anim Hosp Assoc. 38:467-477.

Ljunggren G, 1967. Legg-Perthes disease in the dog. Acta Orthop Scand (Suppl) 95:1-79.

Lynch M, Walsh B, 1998. Genetics and analysis of quantitative traits. Sunderland (MA): Sinauer Associates.

Robinson R, 1992. Legg-Calve-Perthes disease in dogs: Genetic aetiology. J Small Anim Pract. 33:275-276.

Smith KW, 1971. Legg-Perthes' Disease. Vet Clin N Am. 1(3):479-487.

Starr AN, Famula TR, Markward NJ, Baldwin JV, Fowler KD, Klumb DE, Simpson NL, Murphy KE (2007). Hereditary evaluation of multiple developmental abnormalities in the Havanese dog breed. Journal of Heredity (in press).

Vasseur PB, Foley P, Stevenson S, Heitter D,1989. Mode of inheritance of Perthes' disease in Manchester terriers. Clin Orthop Relat Res. 244:281-292.

Budget

1) Salary: Salary is requested for laboratory personnel for the collection of pedigree information, database assembly, and data analysis.

2) Clinical tests on dogs: Radiographs are required for confirmatory diagnosis of LCPD, but the evaluation for LCPD will have occurred before entry into the Texas A&M University Canine Genetics Research (TAMU-CGR) study.
3) Travel expenses: None. Samples will be submitted by owners directly to TAMU-CGR. There is no expected travel to dog shows to collect samples at this time.
4) Specific and total costs to dog owners participating in the research: Owners will not be charged for participation in the TAMU-CGR study and all effort will be made to spare participants any costs by providing supplies and sample shipping; however, expenses associated with the diagnosis of LCPD by the attending veterinarian are separate.

Timeline

Collection and assembly of pedigrees:	3 months
Pedigree analysis and complex segregation analyses:	1 month

Biographical Sketch - Investigator

NAME Murphy, Keith POSITION TITLE Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Indiana University, Bloomington, IN	B.S.	1982	Microbiology
University of Cincinnati Medical School, Cincinnati, OH	M.S.	1986	Molecular Genetics
Louisiana State University, Baton Rouge, LA	Ph.D.	1989	Genetics

Professional Experience

1989-1991	Postdoctorate in Department of Cell, Molecular and Structural Biology, Northwestern University Medical School, Chicago, Illinois.
1991-1993	Postdoctoral Research Geneticist, USDA-Agricultural Research Service, Arthropod-borne Animal Diseases Research Laboratory, Laramie, Wyoming.
1993-1995	Assistant Professor of Biology, The Citadel, Charleston, South Carolina. Medical University of South Carolina, Adjunct Assistant Professor of Microbiology and Member of Graduate Faculty: The Molecular and Cellular Biology and Pathobiology Training Program.

1996-1998	Assistant Professor of Molecular Genetics, The University of Memphis, Memphis, Tennessee.
1998-1999	Associate Professor of Molecular Genetics (with tenure), The University of Memphis, Memphis, Tennessee.
1999-2005	Associate Professor of Pathobiology, Texas A&M University College of Veterinary Medicine and Biomedical Sciences, College Station, Texas. Faculty of Genetics. Faculty of Biotechnology.
2005-present	Professor of Pathobiology (with tenure), Texas A&M University College of Veterinary Medicine and Biomedical Sciences, College Station, Texas. Faculty of Genetics. Faculty of Biotechnology.

Honors and awards:

1977	Invited participant at Indiana University College Credit for High School Students program
1978	Illinois State Scholar
1978	Who's Who Among American High School Students
1982	University of Cincinnati Medical School Scholarship
1984-1985	Served as President of Graduate Student Association at The University of Cincinnati Medical School
1992	Selected Participant at *Biology of Disease Vectors* course
1997	Early Career Research Award
1998	Selected as one of twelve international scientists to participate in canine genomic mapping meeting sponsored by American Kennel Club-Canine Health Foundation and Nestle Purina
1999	*Sigma Xi* Award for Outstanding Published Manuscript
2000	Participant in Jackson Laboratory Short Course in Medical and Experimental Mammalian Genetics
2005	Pfizer Animal Health Award for Excellence in Research

B. Selected peer-reviewed publications for last three years (in reverse chronological order).

*Past or present graduate student or postdoctoral trainee in my laboratory

*Boggs, R.M., *J.A. Moody, *K.L Tsai and K.E. **Murphy** (2007). Identification, amplification and characterization of the miR-17-92 from canine tissue. *Gene* (in press).

*Tsai, K.L., *L.A. Clark and K.E. **Murphy** (2007). Understanding hereditary diseases using the dog and human as companion model systems. *Mammalian Genome* (in press).

*Starr, A.N., T.R. Famula, N.J. Markward, J.V. Baldwin, K.D. Fowler, D.E. Klumb, N.L. Simpson and K.E. **Murphy** (2007). Hereditary evaluation of multiple developmental abnormalities in the Havanese dog breed. *Journal of Heredity* (in press). (**work featured on cover**).

Kerns, J.A., *E.J. Cargill, *L.A. Clark, S.I. Candille, M. Olivier, G. Lust, S.M. Schmutz, K.E. **Murphy** and G.S. Barsh (2007). Linkage and segregation analysis of black and brindled coat color in domestic dogs. *Genetics* 176: 1679-1689.

*Davidson, A.L., *R.J. Bell, G.E. Lees, C.E. Kashtan, G.S. Davidson and K.E. **Murphy** (2007). Genetic cause of autosomal recessive hereditary nephropathy in the English Cocker Spaniel. *Journal of Veterinary Internal Medicine* 21: 394-401.

*Greer, K.A., *S.C. Canterberry and K.E. **Murphy** (2006). Statistical analysis regarding the effects of height and weight on life span of the domestic dog. *Research in Veterinary Science* 2007 82(2):208-14. Epub 2006 Aug 21.

*Greer, K.A., M.A. Higgins, *M.L. Cox, T.P. Ryan, B.R. Berridge, C.E. Kashtan, G.E. Lees and K.E. **Murphy** (2006). Gene expression analysis in a canine model of X-linked Alport Syndrome. *Mammalian Genome* 17: 976-990.

*Clark, L.A., *J.M. Wahl, C.A. Rees and K.E. **Murphy** (2006). Retrotransposon insertion in *SILV* is responsible for merle patterning of the domestic dog. *Proceedings of the National Academy of Sciences* 103: 1376-1381. (**work highlighted by: a. accompanying *Commentary*; b. mention in This Week in *PNAS* and; c. selection of an image for the cover**).

*Tsai, K.L. and K.E. **Murphy** (2006). Clinical and genetic assessments of hip joint laxity in the Boykin Spaniel. *Canadian Journal of Veterinary Research* 70(2): 148-150.

*Canterberry, S.C., *K.A. Greer, C. Hitte, C. André and K.E. **Murphy** (2005). Aging-associated loci in *Canis familiaris*. *Growth, Development and Aging* 69: 101-113.

*Clark, L.A., *J.M. Wahl, J.M. Steiner, W. Zhou, W. Ji, T.R. Famula, D.A. Williams and K.E. **Murphy** (2005). Linkage analysis and gene expression profile of pancreatic acinar atrophy in the German Shepherd Dog. *Mammalian Genome* 16 (12): 955-962. (**work featured on cover**).

Mateescu, R.G., Z. Zhang, *K.L. Tsai, J. Phavaphutanon, N.I. Burton-Wurster, G. Lust, R. Quaas, K.E. **Murphy**, G.M. Acland and R.J. Todhunter (2005). Analysis of allele fidelity, polymorphic information content, and density of microsatellites in a genome wide screen for hip dysplasia in a cross-breed pedigree. *Journal of Heredity* 96 (7): 847-853.

*Moody, J.A., T.R. Famula, R.C. Sampson and K.E. **Murphy** (2005). Identification of microsatellite markers linked to progressive retinal atrophy in the American Eskimo Dog. *American Journal of Veterinary Research* 66 (11): 1900-1902.

*Clark, L.A., K.M. Credille, K.E. **Murphy** and C.A. Rees (2005). Linkage of dermatomyositis in the Shetland Sheepdog to chromosome 35. *Veterinary Dermatology* 16: 392-394.

*Cargill, E.J., T.R. Famula, R.D. Schnabel, G.M. Strain and K.E. **Murphy** (2005). The color of a Dalmatian's spots: Linkage evidence to support the *Tyrp1* gene. *BMC Veterinary Research* 1: 1-5 (journal is published online only).

Credille, K.M., R. Guyon, C. André, K.E. **Murphy**, K. Tucker, K.F. Barnhart and R.W. Dunstan (2005). Comparative sequence analysis and radiation hybrid mapping of two epidermal type II keratin genes in the dog: keratin 1 and keratin 2e. *Cytogenetic and Genome Research* 108: 328-332.

*Clark, L.A., *K.L. Tsai, J.M. Steiner, D.A. Williams. T. Guerra, E.A. Ostrander, F. Galibert and K.E. **Murphy** (2004). Chromosome-specific microsatellite multiplex sets for linkage studies in the domestic dog. *Genomics* 84: 550-554.

*Clark, L.A., T.R. Famula and K.E. **Murphy** (2004). A rapid, single multiplex microsatellite-based assay for use in canine forensics. *American Journal of Veterinary Research* 65 (10): 1446-1450.

*Cargill, E.J, R.D. Schnabel and K.E. **Murphy** (2004). Assignment of canine MSS1 microsatellite markers to chromosomes by linkage. *DNA Sequence* 15: 209-212.

*Cargill, E.J., T.R. Famula, G.M. Strain and K.E. **Murphy** (2004). Heritability and segregation analysis of deafness in US Dalmatians. *Genetics* 166: 1385-1393.

C. Research Support. Selected ongoing or completed (during the last three years) research projects (federal and non-federal support).

1. Lees, G.E. (Co-PI), K.E. Murphy (Co-PI). Gene transfer therapy for Alport syndrome. 2003-2008.
2. Gustafson, T.G. and K.E. Murphy (mentor/sponsor). Cancer: an age-related disease of DNA repair deficiency. Predoctoral Fellow. Howard Hughes Medical Institute. 2004-2008.
3. Clark, L.A., K.E. Murphy and C.A. Rees (Co-Is). Dermatomyositis in the Collie. Canine Health Foundation. 2007-2008.
4. Clark, L.A., K.E. Murphy and C.A. Rees (Co-Is). Investigation of candidate genes for dermatomyositis. American Shetland Sheepdog Association. 2006-2007.
5. Clark, L.A., K.E. Murphy and C.A. Rees (Co-Is). Analysis for linkage disequilibrium with dermatomyositis of the Collie. Collie Health Foundation. 2006-2007.
6. Murphy, K.E. Investigation of genes identified by a canine-specific microarray may support a deficiency in cholesterol biosynthesis in the Havanese. Canine Health Foundation. 2006-2007.
7. Clark, L.A. and K.E. Murphy (Co-Is). The gene for harlequin. Great Dane Charitable Trust. 2006-2007.
8. Canterberry, S.C. and K.E. Murphy (Co-Is). The role of genetics in the widely differing life spans of the domestic dog. Canine Health Foundation. 2006.
9. Murphy, K.E. Investigation of genes identified by a canine-specific microarray may support a deficiency in cholesterol biosynthesis in the Havanese. Canine Health Foundation. 2006-2007.
10. Murphy, K.E. Cholesterol in the Havanese: Examination of dehydrocholesterol reductase-7 as a cause of symptoms similar to Smith-Lemli-Opitz Syndrome and use of a canine microarray to assess gene expression in affected dogs. Havanese Eye Angels Rescue Team. 2004-2006.
11. Murphy, K.E. (PI) and L.A. Clark. Analysis of a candidate gene for pancreatic acinar atrophy in the German Shepherd Dog. Canine Health Foundation. 2004-2005.
12. Murphy, K.E. Pilot Study: Genetics of post-squalene cholesterol biosynthesis in the domestic dog: possible roles in developmental abnormalities. Canine Health Foundation. 2004-2005.
13. Murphy, K.E. Genetics of post-squalene cholesterol biosynthesis in the Havanese, Akita and Samoyed: are defects in this pathway responsible for an array of developmental abnormalities? Havanese Club of America. 2003-2004.
14. Murphy, K.E. Multiplexing of canine minimal screening set 2. Canine Health Foundation. 2003-2004.
15. Bell, R.J. and K.E. Murphy (mentor/sponsor). Graduate Merit Fellowship. Texas A&M University. 2003-2004.

16. Moody, J.A. and K.E. Murphy (mentor/sponsor). Graduate Merit Fellowship. Texas A&M University. 2003-2004.
17. Herbst, S.M. and K.E. Murphy (mentor/sponsor). Pathways to the Doctorate Fellowship. Texas A&M University. 2003-2004.
18. Greer, K.A. and K.E. Murphy (mentor/sponsor). Understanding genetics of aging: *Canis familiaris* model. Individual National Research Service Award (postdoctoral). NIH-NIA. 2003-2005.
19. Murphy, K.E. (PI) and K.A. Greer. Transmission analysis of breed-specific necrotizing encephalitis in the Pug Dog. Canine Health Foundation. 2003-2004.
20. Murphy, K.E. Whole genome screen for analysis of progressive retinal atrophy in the American Eskimo Dog. North American Eskimo Dog Association. 2003-2004.

Biographical Sketch - Investigator

NAME Tsai, Kate POSITION TITLE Assistant Research Scientist

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Texas A&M University, College Station, TX	B.S.	2001	Genetics
Texas A&M University, College Station, TX	Ph.D.	2005	Canine Genetics

Professional Experience

2005- 2006 Post-Doctoral Scientist, Department of Veterinary Science, University of Texas M.D. Anderson Cancer Center-Bastrop, TX

2006- Present Assistant Research Scientist, Department of Pathobiology (VTPB), Texas A&M University (TAMU)-College Station, TX

Award

Phi Eta Sigma Honor Society – TAMU, 1999-present
Fisher Institute Medical Research Award - TAMU College of Veterinary Medicine, 2005

Publications

Tsai KL, Guyon R and Murphy KE. (2003) Identification of isoforms and RH mapping of canine KIT. *Cytogenetic and Genome Research* 102: 261-263.

Greer KA, Cargill EJ, Cox ML, Clark LA, **Tsai KL**, Credille KM, Dunstan RW, Venta PJ and Murphy KE. (2003) Digging up the canine genome- a tale to wag about. *Cytogenetic and Genome Research* 102(104): 249-253.

Clark LA*, **Tsai KL***, Steiner JM, Williams DA, Guerra T, Ostrander EA, Galibert F and Murphy KE. (2004) Chromosome-specific microsatellite multiplex sets for linkage studies in the domestic dog. *Genomics* 84: 550-554. (*these authors contributed equally to this work)

Tsai KL and Murphy KE. (2006) Clinical and genetic assessments of hip joint laxity in the Boykin Spaniel. *Canadian Journal of Veterinary Research* 70(2): 148-150.

Mateescu R, Zhang Z, **Tsai K**, Phavaphutanon J, Burton-Wurster NI, Lust G, Richard Quaas, Murphy KE, Acland GM and Todhunter RJ. (2006) Analysis of allele fidelity, polymorphic information content, and density of microsatellites in a genome wide screen for hip dysplasia in a cross-breed pedigree. *Journal of Heredity* 96(7): 847-853.

Wang-Johanning F, Liu J, Rycaj K, Huang M, **Tsai K**, Rosen DG, Chen D, Lu DW, Barnhart K and Johanning GL. (2006) Expression of multiple human endogenous retrovirus surface envelope proteins in ovarian cancer. *International Journal of Cancer* 120(1): 81-90.

Tsai KL, Clark LA and Murphy KE. (2007) Understanding hereditary diseases using the dog and human as companion model systems. *Mammalian Genome* (in press).

Boggs, R.M., J.A. Moody, **K.L Tsai** and K.E. Murphy (2007). Identification, amplification and characterization of the miR-17-92 from canine tissue. *Gene* (in press).

Book Chapter

Mateescu RG, **Tsai KL**, Zhang Z, Burton-Wurster NI, Lust G, Dykes NL, Acland GM, Quaas RL, Murphy KE, Todhunter R. (2005) QTL Mapping Using Cross Breed Pedigrees: Strategies for Canine Hip Dysplasia. In: The Dog and Its Genome. Ed: E.A. Ostrander, U. Giger, K. Lindblad-Toh. Woodbury, New York. *Cold Spring Harbor Laboratory Press* 407-438.

Presentation

2007 Saluki Club of America National Specialty, Lexington, KY

Extramural Funding

2007 **Tsai KL**, Boggs RM. MicroRNA expression in the domestic dog and its role in canine cancer. Funded by the Canine Health Foundation. ($9,828).

2007 Clark LA, **Tsai KL**. Analysis of degenerative myelopathy in the German Shepherd Dog using the SNP Array. Funded by the Canine Health Foundation. ($12,839).

2007 Clark LA, **Tsai KL**. Sequence analysis of DLA-DRB1 in German Shepherd Dogs having degenerative myelopathy. Funded by the Canine Health Foundation. ($2,160).

Biographical Sketch – Investigator

NAME Starr, Alison POSITION TITLE Graduate Research Assistant

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Clemson University, Clemson, SC	B.S.	2003	Animal Science
Texas A&M University, College Station, TX	Ph.D.	2007	Canine Genetics

Professional Experience

2003- Present Graduate Research Assistant, Department of Pathobiology (VTPB), Texas A&M University (TAMU)-College Station, TX

Honors and Awards

Calhoun Honors College – Clemson University, 1999-2002
Palmetto Fellows – State of South Carolina, 1999-2003
South Carolina Livestock Producer's Award – SC Livestock Producer's Association, 1999-2003
Dean's List – Clemson University, 1999, 2001, 2002
President's List – Clemson University, 2000, 2003

Publications

Moody JA and **Starr AN** (2007). Healthcare: Merle Coat Pattern. *Continental Kennel Club Magazine* 3(1): 28-29. (non-peer reviewed).

Starr AN, Famula TR, Markward NJ, Baldwin JV, Fowler KD, Klumb DE, Simpson NL and Murphy KE (2007). Hereditary evaluation of multiple developmental abnormalities in the Havanese dog breed. *Journal of Heredity* (in press).

Presentations

2004 Havanese Club of America National Specialty, Houston, TX
2005 Student Research Week, College Station, TX
2005 Havanese club of America Blue Ridge Specialty, Lexington, VA
2005 Third International Symposium on the Genetics of Animal Health, Ames, IA
2005 Havanese Club of America National Specialty, Chicago, IL
2006 Third International Conference on Advances in Canine and Feline Genomics, Davis, CA
2006 Havanese Club of America National Specialty, Richmond, VA
2006 Continental Kennel Club, Denham Springs, LA
2007 American Kennel Club and AKC Canine Health Foundation Breeder's Symposium, Springfield, MO
2007 Havanese Club of America National Specialty, Denver, CO